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(54) Title: PERSONAL CARE COMPOSITIONS CONTAINING ACTIVE PROTEINS TETHERED TO A WATER INSOLUBLE SUBSTRATE (57) Abstract The present invention relates to personal care compositions comprising a water insoluble substrate, a plurality of active proteins, and a binding means, comprising a polymeric tether, permanently attaching each of the enzymes to the substrate wherein the personal care composition comprises from about 0.01 $\mu\text{g}/\text{cm}^2$ to about 1000 $\mu\text{g}/\text{cm}^2$ of the enzyme on the substrate.		

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BACKGROUND OF THE INVENTION

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It is also realized, however, that many active proteins, including enzymes, are potential antigens, and may cause allergic reactions in humans under certain conditions. The human immune system can produce specific antibodies upon exposure to active proteins. This process of producing specific antibodies is referred to as "immunization" when a clinically beneficial response is obtained. When the response leads to hypersensitivity, however, it is referred to as "sensitization". Allergenic sensitization to active proteins has been observed in environments where humans are regularly exposed to the protein. Such environments include manufacturing facilities, where workers can be exposed to uncontrolled dust or aerosol containing an active protein, or the marketplace, where consumers' repeated use of products containing active proteins has, on occasion, caused an allergic reaction.

Presently, allergic responses to active proteins can be minimized by limiting the selection of those proteins used in products to those of human origin. While this approach minimizes allergenicity problems, it is not a complete solution since it is often not possible to find such an active protein which also has the activity properties desired.

Another way of diminishing allergic response has been to reduce the size of the protein molecules (see JP Patent Publication Number 4,112,753). However, size reduction may also cause a significant reduction in biological activity.

A third proposition for decreasing allergenicity is through epitope mapping and alteration of the protein amino acid sequence to deliver a protein with reduced allergenicity. This approach usually requires a large investment of development time and money.

In the medical field, suggestions have been made to diminish the immunogenicity of proteins through yet another method. This method involves attaching unreactive polymers to the protein. U.S. Patent No. 4,179,337 (Davis, et al.) relates to enzymes coupled to substantially straight chain polyethylene glycol (PEG) or polypropylene glycol (PPG) polymer moieties. While PEG/PPG coupling was found to mitigate the allergenicity of the enzyme, only 15% of the physiological activity was maintained. PCT Application WO 96/17929 (Olsen, et al., published June 13, 1996) relates to the modification of enzymes by conjugating them with suitable polymers. The Olsen application describes modified enzymes which demonstrate a reduction in allergenicity of from 25% to 66% compared to the parent enzyme, while maintaining from 39% to 100% of the activity of the parent.

The U. S. Patent Application, Serial Number 08/903,298 discloses the use of enzymes modified by the addition of twin polyethylene glycol polymer moieties to reduce allergenicity while delivering high enzymatic activity. The modified enzyme therein is used in combination with a fibrous substrate in a wipe application. The modified enzymes are not attached to the substrate. Reduced allergenicity is achieved via the modification of the enzyme.

The U. S. Patent Application, Serial Number 09/088,912 disclosed polymeric chemical modification of subtilisin enzymes at one or more of three specific epitope regions which were found to mask the immunogenic determinants of the enzyme.

Another approach to reduce the allergenicity of active proteins has been by granulating, coating or dissolving the active proteins to avoid their becoming airborne. U.S. Patent 4,556,554 (Calvo) discloses cosmetic compositions which comprise enzymes which have been immobilized by attachment to particles of polymeric support. The particles with attached enzymes are dispersed in the cosmetic vehicle. Upon application of the vehicle to the skin, the enzyme is released from the support and is therefore reactivated. Methods such as this address consumer exposure to airborne proteins, however they still leave the substantial risks associated with extended tissue contact with the released enzyme which are deposited on the skin.

Canadian Patent 1,229,808, issued December 1, 1987 teach the immobilization of enzymes, specifically β -galactosidase and β -glucosidase, on cellulosic substrates wherein the enzyme is immobilized by absorption into a agarose gel coating the substrate.

UK Patent Application GB 2,240,040, published July 24, 1991 also teaches immobilized enzymes on substrates. Enzymes, therein as covalently bonded to substrates to provide a medicated dressing.

The activity of enzymes used in biological equipment such as biosensors, bioseparators, and bioreactors has been enhanced by the use of site-specific attachment of enzymes to equipment surfaces. See Huang et al., "Improving the Activity of Immobilized Subtilisin by Site-specific Attachment to Surface", Analytical Chemistry, 69(22), November 15, 1997. Huang teaches the immobilization of subtilisin enzymes via mutation of serine249 or serine145 to cysteine, and bonding to silica beads functionalized with amino groups.

It would be highly desirable to develop a composition which would provide improved levels of protein activity while maintaining low allergenic responses from exposure to the active proteins. If this were accomplished it would provide consumers with safer ways to utilize the benefits of protein technology.

It is an object of the present invention to provide a personal care composition which delivers this activity while maintaining reduced stimulation of and resulting activation of the immune system. It is also an object of the present invention to provide this biological activity, with reduced risk of allergic reaction in the form of a personal care wipe or a personal care skin mask.

SUMMARY OF THE INVENTION

The present invention relates to personal care compositions comprising a water insoluble substrate, a plurality of active proteins, and a binding means, comprising a polymeric tether,

permanently attaching each of the proteins to the substrate wherein the personal care composition comprises from about 0.01 $\mu\text{g}/\text{cm}^2$ to about 1000 $\mu\text{g}/\text{cm}^2$ of the protein on the substrate.

DETAILED DESCRIPTION OF THE INVENTION

5 The personal care compositions of the present invention comprise active proteins permanently bound to a water insoluble substrate with a polymeric tether. The compositions are highly efficacious for cleaning sweat, sebum, dead skin cells, fats and oils from the skin and for moisturizing of the skin. The compositions provide a convenient means to utilize the specialized activity of enzymes and other active proteins. The active proteins are bound to the substrate, thereby minimizing any risk of allergic reaction. By attaching the active proteins to the substrate
10 by way of a polymeric tether the active proteins are more mobile on the surface of the substrate, thereby allowing the proteins easier access to dirt and dead skin cells.

Without being limited by theory, it is believed that by permanently binding the active protein to the substrate, the plurality of proteins may be brought into contact with the skin for use, allowing them to act on the surface. Then as the wipe is removed all of the proteins are
15 lifted from the skin surface, and removed and disposed of with the used wipe, thereby eliminating the risk of aerosolization and extended dermal exposure. The active protein reacts with the compounds it has specific reactivity for while in contact with the skin and none remain on the skin after use to stimulate the immune system and subsequently form antibodies responsible for
20 allergic reaction.

As used herein, the phrase "amino acid sequence" refers to a specific configuration of the amino acids comprising a protein. The following is a list of abbreviations used herein to describe amino acids:

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
25	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic Acid	Asp	D
	Cysteine	Cys	C
30	Glutamine	Gln	Q
	Glutamic Acid	Glu	Q
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
35	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
40	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y

Valine	Val	V
No amino acid at position	Xaa	*

As used herein, the term "mutation" refers to the genetic alteration of an organism, which in turn alters the amino acid sequence of the enzyme produced by that organism. The mutation of an organism has been often found to alter the properties of the enzyme.

As used herein, the term "wild-type" refers to an enzyme produced by unmutated hosts.

As used herein, the term "variant", means an enzyme having an amino acid sequence which differs from that of the wild-type enzyme due to the genetic mutation of the host producing that enzyme.

All percentages and ratios used herein, unless otherwise indicated, are by weight and all measurements made are at 25°C, unless otherwise designated. The invention hereof can comprise, consist of, or consist essentially of, the essential as well as optional ingredients and components described therein.

The essential components of the personal care compositions of the present invention, as well as a non-exclusive list of preferred and optional ingredients, are described in detail below.

WATER INSOLUBLE SUBSTRATE

The products of the present invention comprise a water insoluble substrate. By "water insoluble" is meant that the substrate does not dissolve in or readily break apart upon immersion in water. The water insoluble substrate is the implement or vehicle for delivering the active proteins of the present invention to the skin to be cleansed and moisturized, and for removing substantially all of the proteins from the skin.

A wide variety of materials can be used as the substrate. The following nonlimiting characteristics are desirable: (i) sufficient wet strength for use, (ii) sufficient abrasivity, (iii) sufficient loft and porosity, (iv) sufficient thickness, and (v) appropriate size.

Nonlimiting examples of suitable insoluble substrates which meet the above criteria include nonwoven substrates, woven substrates, hydroentangled substrates, air entangled substrates, natural sponges, synthetic sponges, polymeric netted meshes, and the like. Preferred embodiments employ nonwoven substrates since they are economical and readily available in a variety of materials. By nonwoven is meant that the layer is comprised of fibers which are not woven into a fabric but rather are formed into a sheet, mat, or pad layer. The fibers can either be random (i.e., randomly aligned) or they can be carded (i.e. combed to be oriented in primarily one direction). Furthermore, the nonwoven substrate can be composed of a combination of layers of random and carded fibers.

Nonwoven substrates may be comprised of a variety of materials both natural and synthetic. By natural is meant that the materials are derived from plants, animals, insects or byproducts of plants, animals, and insects. By synthetic is meant that the materials are obtained primarily from various man-made materials or from natural materials which have been further

altered. The conventional base starting material is usually a fibrous web comprising any of the common synthetic or natural textile-length fibers, or mixtures thereof.

Nonlimiting examples of natural materials useful in the present invention are silk fibers, keratin fibers and cellulosic fibers. Nonlimiting examples of keratin fibers include those selected from the group consisting of wool fibers, camel hair fibers, and the like. Nonlimiting examples of cellulosic fibers include those selected from the group consisting of wood pulp fibers, cotton fibers, hemp fibers, jute fibers, flax fibers, and mixtures thereof.

Nonlimiting examples of synthetic materials useful in the present invention include those selected from the group consisting of acetate fibers, acrylic fibers, cellulose ester fibers, modacrylic fibers, polyamide fibers, polyester fibers, polyolefin fibers, polyvinyl alcohol fibers, rayon fibers, polyurethane foam, and mixtures thereof. Examples of some of these synthetic materials include acrylics such as acrilan, creslan, and the acrylonitrile-based fiber, orlon; cellulose ester fibers such as cellulose acetate, arnel, and acele; polyamides such as nylons (e.g., nylon 6, nylon 66, nylon 610, and the like); polyesters such as fortrel, kodel, and the polyethylene terephthalate fiber, dacron; polyolefins such as polypropylene, polyethylene; polyvinyl acetate fibers; polyurethane foams and mixtures thereof. These and other suitable fibers and the nonwoven materials prepared therefrom are generally described in Riedel, "Nonwoven Bonding Methods and Materials," Nonwoven World (1987); The Encyclopedia Americana, vol. 11, pp. 147-153, and vol. 26, pp. 566-581 (1984); U.S. Patent No. 4,891,227, to Thaman et al., issued January 2, 1990; and U.S. Patent No. 4,891,228 which are all incorporated by reference herein in their entirety.

Nonwoven substrates made from natural materials consist of webs or sheets most commonly formed on a fine wire screen from a liquid suspension of the fibers. See C.A. Hampel et al., The Encyclopedia of Chemistry, third edition, 1973, pp. 793-795 (1973); The Encyclopedia Americana, vol. 21, pp. 376-383 (1984); and G.A. Smook, Handbook of Pulp and Paper Technologies, Technical Association for the Pulp and Paper Industry (1986); which are incorporated by reference herein in their entirety.

Substrates made from natural materials useful in the present invention can be obtained from a wide variety of commercial sources. Nonlimiting examples of suitable commercially available paper layers useful herein include Airtex®, an embossed airlaid cellulosic layer having a base weight of about 71 gsy, available from James River, Green Bay, WI; and Walkisoft®, an embossed airlaid cellulosic having a base weight of about 75 gsy, available from Walkisoft U.S.A., Mount Holly, NC.

Methods of making nonwoven substrates are well known in the art. Generally, these nonwoven substrates can be made by air-laying, water-laying, meltblowing, coforming, spunbonding, or carding processes in which the fibers or filaments are first cut to desired lengths

from long strands, passed into a water or air stream, and then deposited onto a screen through which the fiber-laden air or water is passed. The resulting layer, regardless of its method of production or composition, is then subjected to at least one of several types of bonding operations to anchor the individual fibers together to form a self-sustaining web. In the present invention the nonwoven layer can be prepared by a variety of processes including hydroentanglement, thermally bonding or thermo-bonding, and combinations of these processes. Moreover, the substrates of the present invention can consist of a single layer or multiple layers. In addition, a multilayered substrate can include films and other nonfibrous materials.

Nonwoven substrates made from synthetic materials useful in the present invention can also be obtained from a wide variety of commercial sources. Nonlimiting examples of suitable nonwoven layer materials useful herein include HEF 40-047, an apertured hydroentangled material containing about 50% rayon and 50% polyester, and having a basis weight of about 43 grams per square yard (gsy), available from Veratec, Inc., Walpole, MA; HEF 140-102, an apertured hydroentangled material containing about 50% rayon and 50% polyester, and having a basis weight of about 56 gsy, available from Veratec, Inc., Walpole, MA; Novonet® 149-616, a thermo-bonded grid patterned material containing about 100% polypropylene, and having a basis weight of about 50 gsy, available from Veratec, Inc., Walpole, MA; Novonet® 149-801, a thermo-bonded grid patterned material containing about 69% rayon, about 25% polypropylene, and about 6% cotton, and having a basis weight of about 75 gsy, available from Veratec, Inc. Walpole, MA; Novonet® 149-191, a thermo-bonded grid patterned material containing about 69% rayon, about 25% polypropylene, and about 6% cotton, and having a basis weight of about 100 gsy, available from Veratec, Inc. Walpole, MA; HEF Nubtex® 149-801, a nubbed, apertured hydroentangled material, containing about 100% polyester, and having a basis weight of about 70 gsy, available from Veratec, Inc. Walpole, MA; Keybak® 951V, a dry formed apertured material, containing about 75% rayon, about 25% acrylic fibers, and having a basis weight of about 43 gsy, available from Chicopee, New Brunswick, NJ; Keybak® 1368, an apertured material, containing about 75% rayon, about 25% polyester, and having a basis weight of about 39 gsy, available from Chicopee, New Brunswick, NJ; Duralace® 1236, an apertured, hydroentangled material, containing about 100% rayon, and having a basis weight from about 40 gsy to about 115 gsy, available from Chicopee, New Brunswick, NJ; Duralace® 5904, an apertured, hydroentangled material, containing about 100% polyester, and having a basis weight from about 40 gsy to about 115 gsy, available from Chicopee, New Brunswick, NJ; Sontaro 8868, a hydroentangled material, containing about 50% cellulose and about 50% polyester, and having a basis weight of about 60 gsy, available from Dupont Chemical Corp.

Alternatively, the water insoluble substrate can be a polymeric mesh sponge as described in European Patent No. EP 702550 A1 published March 27, 1996, incorporated by reference herein

in its entirety. The polymeric sponge comprises a plurality of plies of an extruded tubular netting mesh prepared from a strong flexible polymer, such as addition polymers of olefin monomers and polyamides of polycarboxylic acids. Although these polymeric sponges are designed to be used in conjunction with a liquid cleanser, these types of sponges can be used as the water insoluble substrate in the present invention.

The substrate can be made into a wide variety of shapes and forms including flat pads, thick pads, thin sheets, ball-shaped implements, irregularly shaped implements, and having sizes ranging from a surface area of about a square inch to about hundreds of square inches. The exact size will depend upon the desired use and product characteristics. Especially convenient are square, circular, rectangular, or oval pads having a surface area of from about 1 in² to about 144 in², preferably from about 10 in² to about 120 in², and more preferably from about 30 in² to about 80 in², and a thickness of from about 1 mil to about 500 mil, preferably from about 5 mil to about 250 mil, and more preferably from about 10 mil to about 100 mil.

The water insoluble substrates of the present invention can comprise two or more layers, each having different textures and abrasiveness. The differing textures can result from the use of different combinations of materials or from the use of different manufacturing processes or a combination thereof. A dual textured substrate can be made to provide the advantage of having a more abrasive side for exfoliation and a softer, absorbent side for gentle cleansing. In addition, separate layers of the substrate can be manufactured to have different colors, thereby helping the user to further distinguish the surfaces.

ACTIVE PROTEIN

An essential component of the present invention is a plurality of active proteins. The active proteins are present on the surface of the water insoluble substrate at a level ranging from about 0.01 µg/cm² to about 1000 µg/cm², preferably from about 0.05 µg/cm² to about 100 µg/cm², and most preferably from about 0.1 µg/cm² to about 10 µg/cm².

An active protein is any amino acid based compound that is capable of inducing a human immune response due to exposure to the compound. The active protein will exhibit either biological function, catalytic activity or cosmetic utility or any combination thereof. Nonlimiting examples of active proteins are antibodies, antibody pieces, animal and vegetable proteins such as collagen, and soy, whey or wheat proteins, modified animal or vegetable proteins such as Crotein Q® (stearyl trimonium hydroxyethyl hydrolyzed collagen), and enzymes.

Any active protein can be used in the personal care wipe compositions of the present invention. Preferred active proteins are enzymes. Enzymes for use in the personal care wipe of the present invention may have any activity known to be used for skin care. These include but are not limited to oxidoreductases, such as laccase and superoxide dismutases; Hydrolases, including proteases and lipases; trasnferases such as transglutaminases and isomerases such as

protein disulfide isomerases. Preferred enzymes are selected from the group consisting of lipase and protease enzymes, and mixtures thereof.

Lipase enzymes are classified under the Enzyme Classification number E.C. 3.1.1 (Carboxylic Ester Hydrolases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB). Examples of lipases include lipases derived from the following microorganisms. The indicated patent publications are incorporated herein by reference:

Humicola, (U.S. 4,810,414)

Pseudomonas (WO 89/04361, U.S. 4,950,417, EP 218 272, WO 88/09367, U.S. 5,389,536)

10 *Fusarium* (EP 130 064, WO 90/09446)

Mucor (EP 238 023)

Chromobacterium

Aspergillus

Candida (WO 88/02775, WO 94/01541, WO 89/02916)

15 *Geotricum*

Penicillium

Rhizopus

Bacillus (WO 91/16422)

Specific examples of commercial lipases include Lipolase®, Lipolase™ Ultra, Lipozyme®, Palatase®, Novozym435, Lecitase® (all available from Novo Nordisk A/S); Lumafast™ and Lipomax (available from Genencor Int., Inc.).

Protease enzymes are classified under the Enzyme Classification number E.C. 3.4 (Carboxylic Ester Hydrolases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB). Useful proteases are also described in PCT publications: WO 95/30010 published November 9, 1995 by The Procter & Gamble Company; WO 95/30011 published November 9, 1995 by The Procter & Gamble Company; WO 95/29979 published November 9, 1995 by The Procter & Gamble Company. Preferred protease enzymes for use in the personal care wipes herein are subtilisin, chymotrypsin and elastase-type protease enzymes.

Especially preferred for use herein are subtilisin-type protease enzymes. Subtilisin enzymes are naturally produced by *Bacillus alcalophilus*, *Bacillus amyloliquefaciens*, *Bacillus amylosaccharicus*, *Bacillus licheniformis*, *Bacillus lentus* and *Bacillus subtilis* microorganisms.

A particularly preferred subtilisin-type enzyme is bacterial serine protease enzyme, and variants thereof, obtained from *Bacillus amyloliquefaciens*, *Bacillus licheniformis* and/or *Bacillus subtilis*, including Novo Industries A/S Alcalase®, Esperase®, Savinase® (Copenhagen, Denmark), Gist-brocades' Maxatase®, Maxacal® and Maxapem 15® (protein engineered

Maxacal®) (Delft, Netherlands), and subtilisin BPN and BPN', which are commercially available.

Especially preferred are protease enzymes, and variants thereof, obtained from *Bacillus amyloliquefaciens*. One known enzyme is BPN'. The wild-type BPN' from *Bacillus*
 5 *amyloliquefaciens* is characterized by the amino acid sequence:

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	Ala	Gln	Ser	Val	Pro	Tyr	Gly	Val	Ser	Gln	Ile	Lys	Ala	Pro	Ala	Leu	His	Ser	Gln	Gly
										30										40
10	Tyr	Thr	Gly	Ser	Asn	Val	Lys	Val	Ala	Val	Ile	Asp	Ser	Gly	Ile	Asp	Ser	Ser	His	Pro
										50										60
	Asp	Leu	Lys	Val	Ala	Gly	Gly	Ala	Ser	Met	Val	Pro	Ser	Glu	Thr	Asn	Pro	Phe	Gln	Asp
										70										80
	Asn	Asn	Ser	His	Gly	Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly
15										90										100
	Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu	Gly	Ala	Asp	Gly
										110										120
	Ser	Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu	Trp	Ala	Ile	Ala	Asn	Asn	Met	Asp
										130										140
20	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Pro	Ser	Gly	Ser	Ala	Ala	Leu	Lys	Ala	Ala	Val	Asp
										150										160
	Lys	Ala	Val	Ala	Ser	Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Thr	Ser	Gly
										170										180
	Ser	Ser	Ser	Thr	Val	Gly	Tyr	Pro	Gly	Lys	Tyr	Pro	Ser	Val	Ile	Ala	Val	Gly	Ala	Val
25										190										200
	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly	Pro	Glu	Leu	Asp	Val	Met	Ala
										210										220
	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr	Leu	Pro	Gly	Asn	Lys	Tyr	Gly	Ala	Tyr	Asn	Gly	Thr
										230										240
30	Ser	Met	Ala	Ser	Pro	His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn
										250										260
	Trp	Thr	Asn	Thr	Gln	Val	Arg	Ser	Ser	Leu	Glu	Asn	Thr	Thr	Thr	Lys	Leu	Gly	Asp	Ser
										270										275
	Phe	Tyr	Tyr	Gly	Lys	Lys	Gly	Leu	Ile	Asn	Asn	Val	Gln	Ala	Ala	Ala	Gln			

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Variants of BPN' are also useful in the personal care wipe of the present invention. Several related variants, all hereafter referred to as "Protease A", are disclosed in U.S. Patent 5,030,378 (issued to Venegas, July 9, 1991) as characterized by the BPN' amino acid sequence with the following mutations:

- 40 a.) the Gly at position Gly166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala or Glu; the Gly at position Gly169 is replaced with Ser; the Met at position Met222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or
- b.) the Gly at position Gly166 is replaced with Lys and the Met at position Met222 is replaced with Cys; or

c.) the Gly at position Gly160 is replaced with Ala and the Met at position Met222 is replaced with Ala.

Additional variants of BPN', heretoforth referred to as "Protease B", are disclosed by Genencor International, Inc. (San Francisco, California) European Patent EP-B-251,446 (granted December 28, 1994 and published January 7, 1988) as characterized by the wild-type BPN' amino acid with the mutations in one or more of the following amino acids: Tyr21, Thr22, Ser24, Asp36, Ala 45, Ala48, Ser49, Met50, His67, Ser87, Lys94, Val95, Gly97, Ser101, Gly102, Gly103, Ile107, Gly110, Met 124, Gly127, Gly128, Pro129, Leu135, Lys170, Tyr171, Pro172, Asp197, Met 199, Ser 204, Lys213, Tyr214, Gly215, and Ser221; or two or more of the amino acids listed above and Asp32, Ser33, Tyr104, Ala152, Asn155, Glu156, Gly166, Gly169, Phe189, Tyr217, and Met222 wherein both mutations cannot be made on the Asp32, Ser33, Tyr104, Ala152, Asn155, Glu156, Gly166, Gly169, Phe189, Tyr217, and Met222 amino acids.

Another preferred BPN' variant protease, hereafter referred to as "Protease D", is described in WO 95/10615 published April 20, 1995 by Genencor International as characterized by the wild-type BPN' amino acid with mutation to position Asn76, in combination with mutations in one or more other amino acid positions selected from the group consisting of Asp99, Ser101, Gln103, Tyr104, Ser105, Ile107, Asn109, Asn123, Leu126, Gly127, Gly128, Leu135, Glu156, Gly166, Glu195, Asp197, Ser204, Gln206, Pro210, Ala216, Tyr217, Asn218, Met222, Ser260, Lys265, and/or Ala274.

Another preferred BPN' variant protease, hereafter referred to as "Protease F", is described in U.S. Patent Number 4,760,025, issued to Estell, et al. on July 26, 1988 as characterized by the wild-type BPN' amino acid with mutation to one or more amino acid positions selected from the group consisting of Asp32, Ser33, His64, Tyr104, Asn155, Glu156, Gly166, Gly169, Phe189, Tyr217, and Met222.

Preferred proteolytic enzymes, then, are selected from the group consisting of Alcalase®, BPN', Protease A, Protease B, Protease D, Protease F, and mixtures thereof.

An additional class of active proteins are "singularly substituted proteins", those proteins wherein the parent amino acid sequence is substituted at one of the amino acid residues with a substitute amino acid which is not present elsewhere in the parent amino acid. Singularly substituted proteins also encompass those proteins wherein one amino acid which is not present in the parent amino acid is inserted into the amino acid sequence. The substituted or inserted amino acid provides a moiety suitable for attachment to the substrate of the present invention at a specific site within the protein.

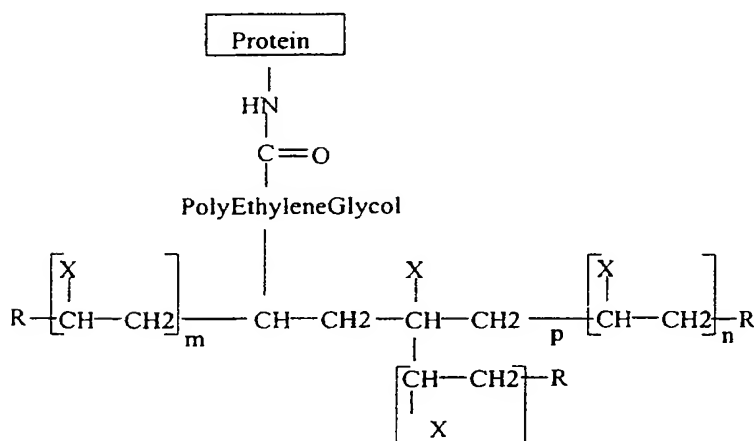
Preferably the substitution or insertion should be made at a position in an epitope region which falls at a point in the protein away from the active site of the protein. One embodiment of these singularly substituted proteins, hereinafter referred to as Protease G, is a modification of subtilisin BPN' and its derivatives. The active site in subtilisin BPN' type enzymes is defined by the spacial triad of Asn32, His64, and Ser 221. In a Protease G enzyme, a cysteine is substituted or inserted at a point away from that triad. Preferable epitope regions for substitution are the of Asp140-Val150 and Ala230-Leu250 region. Non-limiting examples of possible cysteine substitutions at either Ser145, Asn 240 or Ser249. Cysteine is the most preferred substituting amino acid for substitution in the desired epitope region since it does not occur in wild-type subtilisin BPN' or its derivatives.

BINDING MEANS

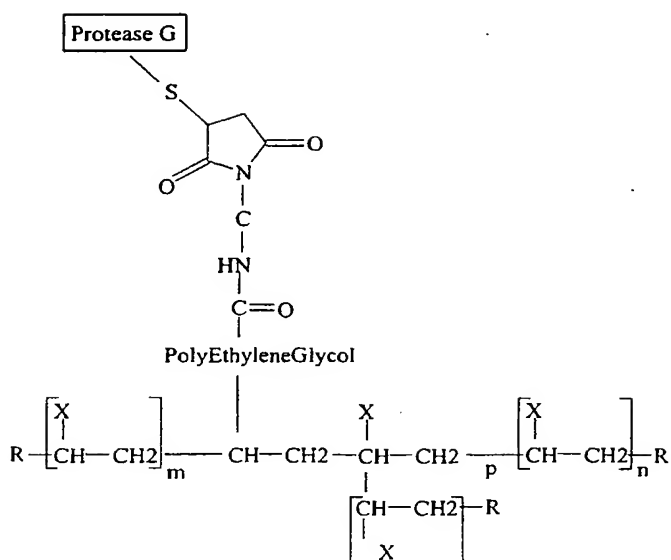
The active proteins are bound to the water insoluble substrate by any suitable binding means which comprises a polymeric tether. Binding means include any chemical method of permanently binding an active protein to a substrate.

Protein Tethered to Polymeric Gel Coating on Substrate

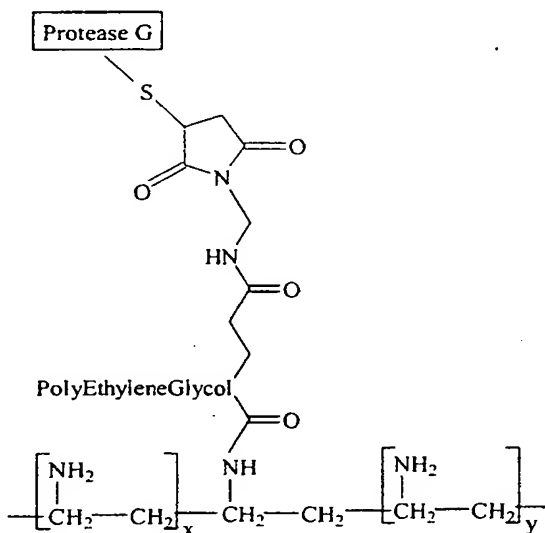
The protein may be bound to the substrate by a polymeric tether bound to a polymeric coating on the substrate. The protein is bonded to a polymeric tether which is covalently attached to the polymer coating. One embodiment is the use of polyethylene glycol (PEG)-N-hydroxy succinimide as the polymeric tether bound to an poly-2-hydroxyethyl acrylate coating.



Another embodiment of a polymeric tether is Polyethylene glycol (PEG)-Maleimide, sold by Shearwater Polymers, Inc. PEG-Maleimide must be used with a cysteine amino acid, therefore it may be used when the active protein is Protease G. PEG-Maleimide may also be used in conjunction with a poly-2-hydroxyethyl acrylate coating. A generic structure of a preferred acrylate-PEG-Maleimide tether can be represented by the formula:



Yet another embodiment of a cysteine containing protein tethered to a polymeric coating on the wipe substrate comprises a PEG-Maleimide covalently bonded to a polyethyleneimine coating by N-hydroxysuccinimide (NHS) as represented by the formula:

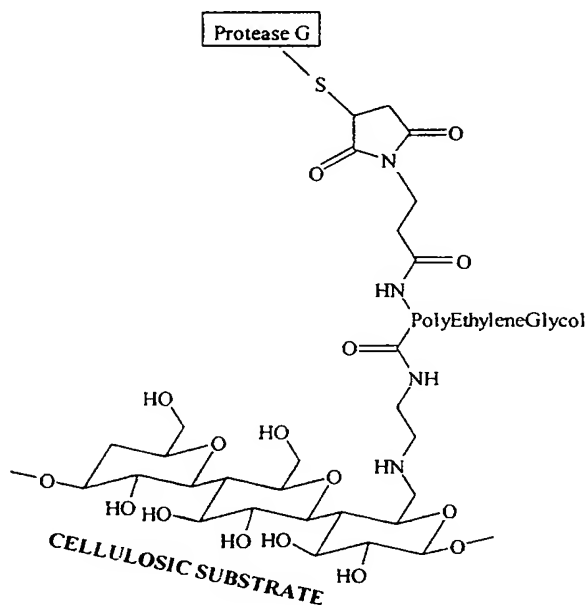


Binding means comprising tethered proteins are preferred over physical entrapment and direct covalent attachment since tethered proteins are more mobile and are less covered by the polymer coating, both of which provide more activity of the proteins.

Protein Covalently Linked to Activated Implement Surface Via Polymeric Tether

Yet another means for binding the active protein to the substrate of the personal care wipe of the present invention is a polymeric tether covalently bonded to an activated site on the surface

of the substrate. The preferred tether is ethylenediamine/polyethylene glycol-Maleimide. A generic structure of a directly bonded ethylenediamine/PEG-Maleimide tether can be represented by the formula:



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OPTIONAL INGREDIENTS

The wipe compositions of the present invention can comprise a wide range of optional ingredients. The CTFA International Cosmetic Ingredient Dictionary, Sixth Edition, 1995, which is incorporated by reference herein in its entirety, describes a wide variety of nonlimiting cosmetic and pharmaceutical ingredients commonly used in the skin care industry, which are suitable for use in the compositions of the present invention. Nonlimiting examples of functional classes of ingredients are described at page 537 of this reference. Examples of these functional classes include: abrasives, anti-acne agents, anticaking agents, anti-microbial agents, antioxidants, binders, biological additives, bulking agents, chelating agents, chemical additives, colorants, cosmetic astringents, cosmetic biocides, denaturants, drug astringents, emulsifiers, external analgesics, film formers, fragrance components, humectants, mildness enhancers (cationic and nonionic polymers, co-surfactants, lipid moisturizers, hydrocarbon oils, silicone oils, waxes), opacifying agents, plasticizers, preservatives, propellants, reducing agents, skin bleaching agents, skin-conditioning agents (emollient, humectants, miscellaneous, and occlusive), skin protectants, solvents, foam boosters, hydrotropes, solubilizing agents, stabilizers, suspending agents, sunscreen agents, surfactants (anionic, cationic, amphoteric, zwitterionic), ultraviolet light absorbers, and viscosity increasing agents (aqueous and nonaqueous). Examples of other

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functional classes of materials useful herein that are well known to one of ordinary skill in the art include solubilizing agents, sequestrants, and keratolytics, and the like.

METHODS OF USE

The personal care compositions of the present invention are useful for personal cleansing, cosmetic skin treatment, and/or skin conditioning. The present invention may take the form of a personal care wipe or a personal care skin mask. Typically, the wipe is used to expose the area to be cleansed to the active enzymes for a relatively short period of time. For use, the wipe is contacted with or wiped skin which needs treatment and then removed. Typical quantities of the present wipes useful for cleansing, range from about 1 to about 4 wipes per use, preferably from about 1 to about 2 wipes per use. The skin mask is used to expose the area to be treated for a relatively longer period of time. Typical quantities of the present skin masks useful for cleansing, range from about 1 to about 2 masks per use, preferably 1 mask per use.

EXAMPLES AND METHODS OF MANUFACTURE

The following examples further describe and demonstrate embodiments within the scope of the present invention. In the following examples, all ingredients are listed at an active level. The examples are given solely for the purpose of illustration and are not to be construed as limitations of the present invention, as many variations thereof are possible without departing from the spirit and scope of the invention. Ingredients are identified by chemical or CTFA name.

The following are nonlimiting examples of the wipes with bound active proteins of the present invention.

EXAMPLE 1 - Protease F tethered to polymeric coating

Purify and concentrate Protease F to approximately 2.5 mg/mL in 10 mM KH_2PO_4 buffer, pH 5.5. Add Acrylate-PEG₃₄₀₀-N-hydroxysuccinimide (Shearwater Polymers, Inc.) in a 15:1 molar excess. Raise the solution pH to 7 with dilute sodium hydroxide (NaOH). Let Protease F and Acrylate-PEG-N-hydroxysuccinimide react for 1-2 hours at room temperature. Drop the solution pH to 5.5 with dilute phosphoric acid (H_3PO_4). Add 2-hydroxyethyl acrylate (HEA) to the Protease F-PEG-N-hydroxysuccinimide solution to achieve a final molar concentration of 1.3 M. Add 50 mM H_2O_2 solution to the Protease F-PEG/HEA solution such that a final molar concentration of 2.5 mM H_2O_2 exists. Spray the solution onto a rayon/PET sheet until the sheet has become saturated. Separately spray 50 mM iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) solution onto the sheet such that the entire surface area is sprayed uniformly. Allow the sheet to sit for 5 minutes. Then rinse the sheet through successive 0.01M KH_2PO_4 baths until all free Protease F has been removed. Dry sheets.

EXAMPLE 2 - Protease G tethered to polymeric coating

Purify and concentrate Protease G to approximately 2.5 mg/mL in 10 mM KH_2PO_4 buffer, pH 5.5. Add Acrylate-PEG₃₄₀₀-Maleimide (Shearwater Polymers, Inc.) in a 15:1 molar excess. Raise the solution pH to 7 with dilute sodium hydroxide (NaOH). Let Protease G and Acrylate-PEG-Maleimide react for 1-2 hours at room temperature. Drop the solution pH to 5.5 with dilute phosphoric acid (H_3PO_4). Add 2-hydroxyethyl acrylate (HEA) to the Protease G-PEG-Maleimide solution to achieve a final molar concentration of 1.3 M. Add 50 mM H_2O_2 solution to the Protease G-PEG/HEA solution such that a final molar concentration of 2.5 mM H_2O_2 exists. Spray the solution onto a rayon/PET sheet until the sheet has become saturated. Separately spray 50 mM iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) solution onto the sheet such that the entire surface area is sprayed uniformly. Allow the sheet to sit for 5 minutes. Then rinse the sheet through successive 0.01M KH_2PO_4 baths until all free Protease G has been removed. Dry sheets.

EXAMPLE 3 - Protease G Covalently Linked to Activated Implement Surface via Polymer Tether

Soak rayon/PET sheets in an aqueous 10% (w/v) NaOH solution for 10-15 minutes with shaking on auto-shaker, using 1mL solution per 1 cm^2 sheet. Wash sheets three times with deionized water under suction ($\sim 1\text{ml}/\text{cm}^2/\text{wash}$). Wash sheets five times with acetone under suction. Allow sheets to soak in acetone for 1 minute between washes. React sheets in a 10% (w/v) p-toluenesulfonyl chloride in acetone solution for 25-30 minutes with shaking ($\sim 1\text{ml}/\text{cm}^2$). Rinse sheets 3 times with acetone under suction to remove excess p-toluenesulfonyl chloride. React sheets in 2.2M ethylenediamine in acetone solution, pH 13-14, for 2 hours with shaking. Rinse sheets 3 times with acetone under suction to remove excess EDA. Rinse sheets 5 times with 0.2M sodium borate buffer, pH 8.5 under suction. Let sheets soak for 1 minute in buffer between rinses. React sheets in 3-5% (w/v) Maleimide-PEG₃₄₀₀-NHS (Shearwater Polymers, Inc.) in 0.2M sodium borate buffer, pH 8.5 for 1-2 hours with shaking ($\sim 1\text{ml}/\text{cm}^2$). Rinse sheets 5 times with 10 mM KH_2PO_4 buffer, pH 7, under suction to remove excess PEG. React sheets in 3 mg/ml Protease G in 10mM KH_2PO_4 buffer, pH 7 ($\sim 1\text{ml}/\text{cm}^2$) for approximately 1-2 hours. Rinse sheets 3 times with 0.01M KH_2PO_4 buffer, pH 5.5. Dry sheets.

EXAMPLE 4 - Protease A Covalently Linked to Activated Implement Surface via Polymer Tether

Soak rayon/PET sheets in an aqueous 10% (w/v) NaOH solution for 10-15 minutes with shaking on auto-shaker, using 1mL solution per 1 cm^2 sheet. Wash sheets three times with deionized water under suction ($\sim 1\text{ml}/\text{cm}^2/\text{wash}$). Wash sheets five times with acetone under suction. Allow sheets to soak in acetone for 1 minute between washes. React sheets in a 10% (w/v) p-toluenesulfonyl chloride in acetone solution for 25-30 minutes with shaking ($\sim 1\text{ml}/\text{cm}^2$).

Rinse sheets 3 times with acetone under suction to remove excess p-toluenesulfonyl chloride. React sheets in 2.2M ethylenediamine in acetone solution, pH 13-14, for 2 hours with shaking. Rinse sheets 3 times with acetone under suction to remove excess EDA. Rinse sheets 5 times with 0.2M sodium borate buffer, pH 8.5 under suction. Let sheets soak for 1 minute in buffer
 5 between rinses. React sheets in 3-5% (w/v) N-hydroxysuccinimide-PEG₃₄₀₀-NHS (Shearwater Polymers, Inc.) in 0.2M sodium borate buffer, pH 8.5 for 1-2 hours with shaking (~1ml/cm²). Rinse sheets 5 times with 10 mM KH₂PO₄ buffer, pH 7, under suction to remove excess PEG. React sheets in 3 mg/ml Protease A in 10mM KH₂PO₄ buffer, pH 7 (~1ml/cm²) for approximately 1-2 hours. Rinse sheets 3 times with 0.01M KH₂PO₄ buffer, pH 5.5. Dry sheets.

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EXAMPLE 5 - Protease G Covalently Linked to Coated Implement Surface via Polymer Tether

Soak rayon/PET sheets in 500 ppm bath of polyethyleneimine for 1 hour with shaking at room temperature (~1-2ml/cm² sheet). Rinse rayon/PET sheets in 2 successive 0.2M sodium borate buffer, pH 12. baths (~5-10 ml/cm²). Rinse sheets in 2 successive 0.2M sodium
 15 buffer, pH 8.5, baths. React sheets in 5 mg/mL NHS-PEG₃₄₀₀-Maleimide in 0.2M sodium borate buffer, pH 8.5, with shaking at room temperature for 1 hour. Rinse cloths through 4 deionized water baths (~5-10 ml/cm²). Rinse cloths through 2 0.01M KH₂PO₄ buffer, pH 7-7.5, baths. Separately, purify and concentrate Protease G to 1-2 mg/ml in 0.01M KH₂PO₄ buffer, pH 7-7.5. React "PEGylated" sheets in Protease G solution, pH 7-7.5, for 1 hour at room temperature with
 20 shaking. Rinse sheets through 5 successive 0.01M KH₂PO₄, pH 5.5, baths to remove unreacted, unbound Protease G. Let sheets dry.

Examples 6-9

25 A personal care wipe composition product is prepared as follows:

<u>Ingredients</u>	<u>Weight Percent</u>			
	Example 6	Example 7	Example 8	Example 9
<u>Phase A</u>				
Water	QS 100	QS 100	QS 100	QS 100
30 Glycerin	10.00	10.00	10.00	10.00
Disodium Lauroamphodiacetate (and) 4.00	4.00	---	---	---
Sodium Trideceth Sulfate				
Sodium Lauroamphoacetate	---	---	2.40	2.40
Sodium Lauroyl Sarcosinate	4.00	4.00	---	---
35 Ammonium Laureth Sulfate	---	---	4.20	4.20
Ammonium Lauryl Sulfate	---	---	1.40	1.40
Polyquarternium-10	0.25	0.25	0.25	0.25

Disodium EDTA	0.10	0.10	0.10	0.10
---------------	------	------	------	------

Phase B

Sucrose Ester Fatty Acid Cottonate	3.00	3.00	3.00	3.00
------------------------------------	------	------	------	------

Petrolatum	----	1.50	----	----
------------	------	------	------	------

5 Cetyl Dimethicone	----	----	----	2.00
---------------------	------	------	------	------

Phase C

Butylene Glycol	2.00	2.00	2.00	2.00
-----------------	------	------	------	------

DMDM Hydantoin (and)	0.20	0.20	0.20	0.20
----------------------	------	------	------	------

Iodopropynyl Carbamate				
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10 Water Insoluble Substrate

A hydroapertured, nonwoven substrate having a basis weight of about 60 gsy comprising 50% rayon and 50% polyester approximately 6 in. by 7.6 in. and a thickness of about 20 mil having a bound active protein per Examples 1-5.

15 In a suitable vessel., the Phase A ingredients are mixed at room temperature to form a dispersion and heated with stirring to 65°C. The Phase B ingredients are mixed in a separate suitable vessel and heated to 65°C. Once the temperatures are the same, the Phase B ingredients are mixed into the vessel containing the Phase A ingredients and then cooled to 45°C. The Phase C ingredients are then mixed together in a separate vessel at room temperature. Next, the Phase C mixture is added into the vessel containing the combination of Phases A and B at room temperature. 1.5 grams of the resulting solution is sprayed onto each substrate. Alternatively, the substrate can be dipped into the resulting solution. The treated substrate is then dried in an oven to constant weight. Alternatively, the treated substrate is dried in a convection oven at 45°C to constant weight.

25 In alternative embodiments, other substrates such as woven substrates, hydroentangled substrates, natural sponges, synthetic sponges, or polymeric netted meshes. Alternative embodiments may be in the form of person care skin masks.

Examples 10-13

A personal care wipe is prepared as follows:

30 IngredientsWeight Percent

	Example 10	Example 11	Example 12	Example 13
--	------------	------------	------------	------------

Phase A

Water	QS 100	QS 100	QS 100	QS 100
-------	--------	--------	--------	--------

Glycerin	10.00	10.00	10.00	10.00
----------	-------	-------	-------	-------

35 Panthenol	0.50	----	0.50	0.50
--------------	------	------	------	------

Sodium Lauroamphoacetate	2.40	2.40	2.40	2.40
--------------------------	------	------	------	------

Ammonium Lauryl Sulfate	1.40	1.40	1.40	1.40
-------------------------	------	------	------	------

	Polyquarternium-10	0.25	0.25	0.25	0.25
	Disodium EDTA	0.10	0.10	0.10	0.10
	<u>Phase B</u>				
	Sucrose Ester Fatty Acid Cottonate	3.00	3.00	3.00	3.00
5	Petrolatum	----	----	----	0.50
	Cetyl Dimethicone	----	----	----	0.50
	Cetyl Ricinoleate	----	2.00	2.00	1.00
	<u>Phase C</u>				
	Butylene Glycol	2.00	2.00	2.00	2.00
10	DMDM Hydantoin (and) Iodopropynyl Carbamate	0.20	0.20	0.20	0.20

Water Insoluble Substrate

A hydroapertured, nonwoven substrate having a basis weight of about 60 gsy comprising 50% rayon and 50% polyester approximately 6 in. by 7.6 in. and a thickness of about 20 mil having a bound active protein per Examples 1-5.

In a suitable vessel., the Phase A ingredients are mixed at room temperature to form a dispersion and heated with stirring to 65°C. The Phase B ingredients are mixed in a separate suitable vessel and heated to 65°C. Once the temperatures are the same, the Phase B ingredients are mixed into the vessel containing the Phase A ingredients and then cooled to 45°C. Next, the Phase C mixture is added into the vessel containing the combination of Phases A and B at room temperature. 1.5 grams of the resulting solution is sprayed onto each substrate. Alternatively, the substrate can be dipped into the resulting solution. The treated substrate is then dried in an oven to constant weight. Alternatively, the treated substrate is dried in a convection oven at 45°C to constant weight.

In alternative embodiments, other substrates such as woven substrates, hydroentangled substrates, natural sponges, synthetic sponges, or polymeric netted meshes. Alternative embodiments may be in the from of person care skin masks.

30 Examples 14-17IngredientsWeight Percent

		Example 14	Example 15	Example 16	Example 17
	<u>Phase A</u>				
	Water	QS 100	QS 100	QS 100	QS 100
35	Disodium Lauroamphodiacetate (and) Sodium Trideceth Sulfate	4.00	4.00	----	----
	Sodium Lauroamphoacetate	----	----	2.40	2.40

	Sodium Lauroyl Sarcosinate	4.00	4.00	----	----
	Ammonium Laureth Sulfate	----	----	4.20	4.20
	Ammonium Lauryl Sulfate	----	----	1.40	1.40
	Disodium EDTA	0.10	0.10	0.10	0.10
5	<u>Phase B</u>				
	Sucrose Ester Fatty Acid Cottonate	3.00	3.00	3.00	3.00
	Petrolatum	----	1.50	----	----
	Cetyl Dimethicone	----	----	----	2.00
	<u>Phase C</u>				
10	DMDM Hydantoin (and) Iodopropynyl Carbamate	0.20	0.20	0.20	0.20
	<u>Water Insoluble Substrate</u>				

A hydroapertured, nonwoven substrate having a basis weight of about 60 gsy comprising 50% rayon and 50% polyester approximately 6 in. by 7.6 in. and a thickness of about 20 mil having a bound active protein per Examples 1-5.

In a suitable vessel., the Phase A ingredients are mixed at room temperature to form a dispersion and heated with stirring to 65°C. The Phase B ingredients are mixed in a separate suitable vessel and heated to 65°C. Once the temperatures are the same, the Phase B ingredients are mixed into the vessel containing the Phase A ingredients and then cooled to 45°C. The Phase C ingredients are then mixed together in a separate vessel at room temperature. Next, the Phase C mixture is added into the vessel containing the combination of Phases A and B at room temperature. 1.5 grams of the resulting solution is sprayed each substrate. Alternatively, the substrate can be dipped into the solution. The treated substrate is then dried in an oven to constant weight. Alternatively, the treated substrate is dried in a convection oven at about 45°C to constant weight.

In alternative embodiments, other substrates such as woven substrates, hydroentangled substrates, natural sponges, synthetic sponges, or polymeric netted meshes. Alternative embodiments may be in the from of person care skin masks.

Examples 18-21

Ingredients

Weight Percent

	Example 18	Example 19	Example 20	Example 21
<u>Phase A</u>				
35 Water	QS 100	QS 100	QS 100	QS 100
Sodium Lauroamphoacetate	2.40	2.40	2.40	2.40
Ammonium Laureth Sulfate	4.20	4.20	4.20	4.20

Ammonium Lauryl Sulfate	1.40	1.40	1.40	1.40
Disodium EDTA	0.10	0.10	0.10	0.10

Phase B

Sucrose Ester Fatty Acid Cottonate	3.00	3.00	3.00	3.00
5 Petrolatum	----	0.50	1.00	----
Cetyl Dimethicone	----	0.50	----	1.00
Cetyl Ricinoleate	2.00	0.50	1.00	1.00

Phase C

10 DMDM Hydantoin (and) Iodopropynyl Carbamate	0.20	0.20	0.20	0.20
---	------	------	------	------

Water Insoluble Substrate

A hydroapertured, nonwoven substrate having a basis weight of about 60 gsy comprising 50% rayon and 50% polyester approximately 6 in. by 7.6 in. and a thickness of about 20 mil having a bound active protein according to Examples 1-5.

15

In a suitable vessel., the Phase A ingredients are mixed at room temperature to form a dispersion and heated with stirring to 65°C. The Phase B ingredients are mixed in a separate suitable vessel and heated to 65°C. Once the temperatures are the same, the Phase B ingredients are mixed into the vessel containing the Phase A ingredients and then cooled to 45°C. Next, the Phase C mixture is added into the vessel containing the combination of Phases A and B at room temperature. 1.5 grams of the resulting solution is sprayed onto each substrate. Alternatively, the substrate can be dipped into the solution. The treated substrate is then dried in an oven to constant weight. Alternatively, the treated substrate is dried in a convection oven at about 45°C to constant weight.

25 In alternative embodiments, other substrates such as woven substrates, hydroentangled substrates, natural sponges, synthetic sponges, or polymeric netted meshes. Alternative embodiments may be in the form of person care skin masks.

WHAT IS CLAIMED IS:

1. A personal care wipe composition characterized in that it comprises:
 - a) a water insoluble substrate,
 - b) a plurality of active proteins, and
 - c) a binding means, comprising a polymeric tether, permanently attaching each of the enzymes to the substrate;wherein the personal care wipe composition comprises from $0.01 \mu\text{g}/\text{cm}^2$ to $1000 \mu\text{g}/\text{cm}^2$ of the enzyme on the substrate.
2. A personal care skin mask composition characterized in that it comprises:
 - a) a water insoluble substrate,
 - b) a plurality of active proteins, and
 - c) a binding means, comprising a polymeric tether, permanently attaching each of the enzymes to the substrate;wherein the personal care wipe composition comprises from $0.01 \mu\text{g}/\text{cm}^2$ to $1000 \mu\text{g}/\text{cm}^2$ of the enzyme on the substrate.
3. A personal care composition according to either of Claim 1 or Claim 2, wherein said water insoluble substrate comprises one or more materials selected from the group consisting of silks, keratins, celluloses, acetates, acrylics, cellulose esters, modacrylics, polyamides, polyesters, polyolefins, polyvinyl alcohols, wood pulp, cotton, hemp, jute, flax, acrylics, nylons, polyesters, polypropylenes, polyethylenes, polyvinyl acetates, polyurethanes, rayon, and mixtures thereof.
4. A personal care wipe composition according to any of the preceding claims wherein said water insoluble substrate comprises a nonwoven sheet of fibers selected from the group consisting of rayon fibers, cellulose fibers, polyester fibers, and mixtures thereof.

5. A personal care wipe composition according to any of the preceding claims wherein said water insoluble substrate comprises two or more sheets of fibers each in turn having different textures.
6. A personal care wipe composition according to any of the preceding claims wherein the active proteins are enzymes selected from the group consisting of protease enzymes, lipase, enzymes, and mixtures thereof.
7. A personal care wipe composition according to any of the preceding claims, wherein the enzyme is selected from the group consisting of subtilisin, chymotrypsin, and elastase-type enzymes, and mixtures thereof.
8. A personal care wipe composition according to any of the preceding claims, wherein the enzyme is selected from the group consisting of Alcalase[®], BPN', Protease A, Protease B, Protease D, Protease F, and mixtures thereof.
9. A personal care wipe composition according to any of the preceding claims, wherein the enzyme is a singularly substituted protein.
10. A personal care wipe composition according to any of the preceding claims, wherein the singularly substituted protein is substituted at a point spacially away from the active site of the protein.
11. A personal care wipe composition according to any of the preceding claims, wherein the singularly substituted protein is Protease G.
12. A personal care wipe composition according to any of the preceding claims, wherein the polymeric tether comprises polyethylene glycol-maleimide.
13. A personal care wipe composition according to any of the preceding claims, wherein the polymeric tether comprises polyethylene glycol-N-hydroxysuccinimide.

14. A personal care wipe composition according to any of the preceding claims, wherein the binding means comprises a polymeric tether and a polymeric coating.
15. A personal care wipe composition according to any of the preceding claims wherein the polymeric tether comprises polyethylene glycol-maleimide and the polymeric coating comprises poly-2-hydroxyethyl acrylate.
16. A personal care wipe composition according to any of the preceding claims, wherein the polymeric tether comprises polyethylene glycol-maleimide and the polymeric coating comprises polyethyleneimine.
17. A personal care wipe composition according to any of the preceding claims, wherein the polymeric tether comprises polyethylene glycol-N-hydroxysuccinimide and the polymeric coating comprises poly-2-hydroxyethyl acrylate
18. A personal care wipe composition according to any of the preceding claims, wherein the polymeric tether comprises polyethylene glycol-N-hydroxysuccinimide and the polymeric coating comprises polyethyleneimine.
19. A method for improving skin condition comprising the contacting of the personal care wipe composition of any of the preceding claims with skin in need of such treatment.



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(21) International Application Number: PCT/US99/21050 (22) International Filing Date: 14 September 1999 (14.09.99) (30) Priority Data: 60/101361 22 September 1998 (22.09.98) US (71) Applicant: THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US). (72) Inventors: WEISGERBER, David, John; 2632 Fairhill Drive, Cincinnati, OH 45239 (US). ALLCOCK, Andrew, Camp- bell; 3837 Drake Avenue, Cincinnati, OH 45209 (US). (74) Agents: REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217-1087 (US).		(81) Designated States: AU, BR, CA, CN, CZ, CZ (Utility model), JP, KR, MX, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> (88) Date of publication of the international search report: 25 May 2000 (25.05.00)
(54) Title: PERSONAL CARE COMPOSITIONS CONTAINING ACTIVE PROTEINS TETHERED TO A WATER INSOLUBLE SUBSTRATE (57) Abstract The present invention relates to personal care compositions comprising a water insoluble substrate, a plurality of active proteins, and a binding means, comprising a polymeric tether, permanently attaching each of the enzymes to the substrate wherein the personal care composition comprises from about 0.01 $\mu\text{g}/\text{cm}^2$ to about 1000 $\mu\text{g}/\text{cm}^2$ of the enzyme on the substrate.		

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CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/21050

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K7/00 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C12N A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 24427 A (PROCTER & GAMBLE) 10 July 1997 (1997-07-10) the whole document	1-19
A	GB 916 931 A (EPHRAIM KATCHALSKI) 30 January 1963 (1963-01-30) example 4	1-19
A	PATENT ABSTRACTS OF JAPAN vol. 015, no. 056 (C-0804), 8 February 1991 (1991-02-08) & JP 02 286085 A (JAPAN STEEL WORKS LTD:THE), 26 November 1990 (1990-11-26) abstract	1-19
	-/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

14 March 2000

Date of mailing of the international search report

20/03/2000

Name and mailing address of the ISA

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Couckuyt, P

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/US 99/21050

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 240 040 A (VNII TEXTIL GALANTEREY PROMY ;MO MED STOMATOLOG (SU)) 24 July 1991 (1991-07-24) cited in the application the whole document	1-4
A	US 4 179 337 A (DAVIS FRANK F ET AL) 18 December 1979 (1979-12-18) cited in the application the whole document	1-4
A	WO 96 17929 A (BECK THOMAS CHRISTIAN ;NOVONORDISK AS (DK); HANSEN LARS BO L (DK);) 13 June 1996 (1996-06-13) cited in the application the whole document	1-4
A	PATENT ABSTRACTS OF JAPAN vol. 012, no. 358 (C-531), 26 September 1988 (1988-09-26) & JP 63 115813 A (NITTO ELECTRIC IND CO LTD), 20 May 1988 (1988-05-20) abstract	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 21050

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-19
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-19

Present claims 1-19 relate to a composition defined by reference to a desirable characteristic or property, namely "water insoluble substrate", "active proteins" and "polymeric tether".

The claims cover all compositions having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compositions. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely the examples disclosed in the description with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/21050

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9724427	A	10-07-1997	AU 4744996 A BR 9510680 A EP 0877787 A JP 11502256 T	28-07-1997 30-03-1999 18-11-1998 23-02-1999
GB 916931	A		NONE	
JP 02286085	A	26-11-1990	JP 1987628 C JP 7016405 B	08-11-1995 01-03-1995
GB 2240040	A	24-07-1991	CS 9000055 A FR 2657015 A DE 4000797 A	13-05-1992 19-07-1991 18-07-1991
US 4179337	A	18-12-1979	CA 1033673 A CH 616942 A DE 2433883 A FR 2313939 A GB 1469472 A JP 1152589 C JP 50042087 A JP 56023587 B NL 7409770 A SE 441753 B SE 7409366 A	27-06-1978 30-04-1980 05-02-1976 07-01-1977 06-04-1977 30-06-1983 16-04-1975 01-06-1981 22-01-1975 04-11-1985 21-01-1975
WO 9617929	A	13-06-1996	AU 697440 B AU 4114496 A BR 9509976 A CA 2206852 A CN 1168694 A EP 0796324 A FI 972443 A JP 10510516 T US 5981718 A US 5856451 A	08-10-1998 26-06-1996 09-06-1998 13-06-1996 24-12-1997 24-09-1997 09-06-1997 13-10-1998 09-11-1999 05-01-1999
JP 63115813	A	20-05-1988	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

BNSDOCID: <WO_0016733A3_1_>